IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: :
MICHAEL J. ADANG and
JOHN D. KEMP :

Serial No.: 07/713,624 : Group Art Unit: 1804

Filed: June 10, 1991 : Examiner: Dr. Che Chereskin

For: INSECT RESISTANT PLANTS :

Attorney Docket No. 7285-012 : 1155 Avenue of the Americas
New York, New York 10036

DECLARATION OF DR. GUY A. CARDINEAU UNDER 37 C.F.R. §1.132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

S I R:

I, GUY A. CARDINEAU, declare and state as follows:

1. I reside at 1013 Tramore Trail, Madison, Wisconsin 53717.

2. I received a Bachelor of Arts degree in American Civilization in 1972 from Lafayette College, a Bachelor of Science in Microbiology from Auburn University in 1977 and a Doctor of Philosophy degree in Microbiology/Molecular Biology in 1983 from the University of Alabama, Birmingham, Alabama. My dissertation was on the characterization of the aspartate B-semialdehyde dehydrogenase gene of *Streptococcus mutans*.

3. Since August, 1989, I have been Senior Research Scientist and Manager of Molecular Biology at the Agrigenetics Company, Madison Laboratories, 5649 East Buckeye Road, Madison, Wisconsin 53716. The Agrigenetics Company is a division of the Lubrizol Corporation, which wholly owns Lubrizol Genetics, Inc., the assignee of the above-captioned application. Prior to August, 1989, I was a scientist in Molecular Biology at Sungene Technologies, Corporation in San Jose, California. My

publications and other professional data are listed in my curriculum vitae, which is annexed hereto as Exhibit 1.

4. I have studied the genes of *Bacillus thuringiensis* and related organisms since 1987. I am an inventor of discoveries in plant molecular biology described in five pending patent applications. Through my years of research and professional activities in this scientific area, I am familiar with the general level of skill of those working in the field of plant molecular biology from 1983 to the present.

5. I have read and am familiar with the invention entitled "Insect Resistant Plants" by Dr. Michael Adang and Dr. John Kemp as described in patent application Serial No. 535,354, filed September 26, 1983 (which I refer to hereafter as the first application), its related CIP application Serial No. 848,733 filed April 4, 1986, and the additional related CIP application Serial No. 07/260,574 filed October 20, 1988 which is the parent of the pending file wrapper continuation application Serial No. 07/713,624 filed on June 20, 1991. I have read and am familiar with the outstanding Office Action in application Serial No. 07/260,574 dated December 11, 1990.

6. I am familiar with the development of the invention of Dr. Michael Adang and Dr. John Kemp, the literature resulting from this invention and the resulting research and commercial embodiments of this invention. I am familiar with the general literature related to the development of transgenic plants expressing insecticidal foreign genes.

7. As described in their patent applications, Dr. Michael Adang and Dr. John Kemp have developed vectors useful for producing novel transgenic plants expressing foreign insecticidal proteins and have stably transformed many different plant species with their vectors. The plant cells, plant tissues and plants of the invention are tested in a variety of ways to ensure they are

stably transformed and express the insecticidal protein gene. Multiple generations of plants have been analyzed to ensure the stability of the integration of the insecticidal protein gene into the genetic material of the plant.

8. Assays are used to detect the presence of the insecticidal protein gene and/or protein in the plant. These assays include a direct analysis of the protein by an immunological determination of the expressed protein using ELISA assays and Western blots. Indirect tests for the presence of the protein by directly analyzing the presence of RNA or DNA encoding the protein is accomplished by methods well known in the art like Northern and Southern hybridization analysis and restriction enzyme analysis. Other tests that indirectly assay the presence of an insecticidal protein gene include the detection of a product of a foreign gene that is co-transformed into the transgenic plant using a chosen vector that contains additional foreign genes, *e.g.* the detection of octopine using vectors containing the synthetase gene for this amino acid or the detection of NPT II, the neomycin phosphotransferase gene, neither of which are expressed in normal plant cells or tissues.

9. The protein that is expressed by the foreign insecticidal protein gene is present in very low amounts in the transgenic plant cells, tissues or plants of the invention. The assay that is most sensitive in detecting the presence of a small amount of active toxic protein in plant samples is the insect bioassay. Plant cells, tissues and plants are tested under both laboratory and field conditions using this sensitive assay.

10. In the insect bioassay, insects like tobacco hornworm or tobacco budworm are allowed to ingest the plant cells, tissue or plants of the invention. The weight, growth and survival of the insects is measured to determine the effect of the expression of the foreign insecticidal protein gene on the

insects. Some plants are also field tested for their ability to resist insect pests that are naturally present in a local environment, rather than artificially supplied by a laboratory investigator.

11. In my professional capacity, I have reviewed the notebooks and records related to the invention described in pending application Serial No. 07/713,624. I have also communicated with Dr. Michael Adang and have obtained information from him regarding his invention. I was asked to obtain as much photographic evidence as possible, from the notebooks or from Dr. Adang, related to the different transgenic plants of the invention in order to demonstrate their health and vitality, as well as to visually record their insecticidal nature. Unfortunately, routine photographs of the various plants were not taken for normal notebook purposes. However, I have been able to find several photographs which are attached hereto as exhibits to my declaration with the notebook assay data related to the plants depicted in the photographs.

12. Exhibit 2 attached hereto is a color photograph demonstrating one type of a routine bioassay experiment for transgenic tobacco plants and its related notebook bioassay data. In this experiment, tobacco seeds were planted and seedlings were moved to trays 25 days later. The bioassays were started an additional 24 days later. As demonstrated in the photo, tobacco hornworm larvae (see arrow) were placed on seedlings for which the largest leaves were approximately 3.0-3.5 cm long. At different time periods thereafter, the insects on each plant were characterized as to weight and mortality. In the photograph of Exhibit 2, the left plant LK5-107 was a control plant resulting from a initial transformation with pH 400 vector only which had no insecticidal protein gene present in the vector construct. The right plant 108-78 was a transgenic plant expressing an

insecticidal protein gene resulting from an initial transformation with a construct having an ORF 24 promoter/crystal protein structural gene/ORF 24 polyadenylation site located between the ocs gene and a plant expressible kan gene. The transformed plant 108-78 and the type of constructs used to create it were developed according to the teachings of the first filed application Serial No. 535,354, as found therein at pages 20-81. The specific plasmid is described in Example §12.6 of the pending specification at page 115.

13. Several points can be made from the photograph of Exhibit 2. First, 108-78 is a healthy plant grown from seed obtained from a parental transformed plant. Second, as noted on the accompanying notebook page and as visually obvious from an inspection of the photograph, insecticidal plant 108-78 has been barely nibbled by its resident larvae in complete contrast to plant LK5-107 which has been decimated by its resident larvae. Third, the resident larvae of insecticidal plant 108-78 are visibly smaller, whiter and more sickly than the resident larvae of plant LK5-107 as apparent by comparing the larvae in the photograph. Fourth, numerous plants were tested as indicated by this single page of the notebook data and numerous plants were demonstrated to be stably transformed plants expressing insecticidal protein genes.

14. It is my belief and opinion that the photograph of Exhibit 2 vividly and visually demonstrates the insect resistant plants of the invention. I have used this photograph in slide form for presentations regarding the novelty and utility of the invention.

15. The second type of bioassay is demonstrated in the photograph of Exhibit 3. For experiments of this type, equivalent amounts of tobacco leaves were placed with larvae on moistened filter papers in petri dishes. Larvae were scored for

mortality and weight. The photograph of Exhibit 3, already of record in this application as Exhibit B of the DECLARATION OF MICHAEL J. ADANG UNDER 37 C.F.R. §132 dated April 12, 1989, and filed with the Preliminary Amendment for application Serial No. 260,574 on or about April 14, 1989, illustrates the contents of two dishes containing intact tobacco leaves labeled 100 on the left and two dishes of tobacco leaf remains labeled 103 on the right. The tobacco leaves of insecticidal plant 100 were obtained from cloned plants resulting from a transformation using a vector containing an ORF 24 promoter/crystal protein structural gene/ORF 24 polyadenylation site located between the ocs gene and a plant expressible kan gene. The constructs used in the transformation, and the methods to obtain the transformed plants, are taught in the specification of application Serial No. 535,354 at pages 20-81. The specific vector is described in Example §12.6 of the specification at page 115. The non-insecticidal leaves of clone 103 were from a chimeric plant and were used as a control, as disclosed in the specification of the pending application at page 124. This photograph not only visually and vividly demonstrates the production of stably transformed insect resistant plants according to the teachings of the invention, but also demonstrates the careful attention to experimental detail that was undertaken as necessary to distinguish stably transformed plants from nontransformed or chimeric plants, such as that producing the noninsecticidal leaves in the dishes labeled 103.

16. Exhibit 4 provides a photographic representation of two specific tomato plants made according to the invention and used in an experiment with the accompanying descriptive notebook pages of the experiment. On the decimated and ravaged control tomato plant UC82 in the left side of the photograph, at least three large fat larvae can be identified amongst the remaining

plant stalks in the major field of the photograph. In contrast, the insecticidal tomato plant 585-13 on the right in the photograph is healthy, shows normal tomato fruit and no visible larvae. The attached notebook pages of the exhibit quantify the visual results. The control plant UC82 had a mean worm weight of 6.55 with a deviation of 0.64 for its eight larvae. Plant 585-13 had a mean worm weight of 3.22 with a deviation of 1.26 representing one dead and nine live but slow growing larvae. The recordation of the photograph is found on the second notebook page of the exhibit where it notes that plant 585-13 was ELISA positive for Bt in accordance with the results of the bioassay. The third notebook page of the exhibit records the positive ELISA results for plant 585-13 in addition to the positive results of many other tested plants.

17. Exhibits 5, 6, and 7 are photographs provided by Dr. Michael Adang demonstrating the results of similar bioassays where control plants are compared with the insecticidal tomato plants made according to the methods of the invention. As taught by the specification of the invention, the insecticidal tomato plant of Exhibit 5 was produced after a transformation event utilizing a mannopine/full length Bt 73 gene vector. The insecticidal tomato plant of Exhibit 6 was produced after a transformation event utilizing a CaMV19S/full length Bt gene vector. The insecticidal tomato plant of Exhibit 7 was produced after a transformation event utilizing a mannopine/dipel 5.3 gene vector. All vectors were produced according to the teachings of the first application Serial No. 535,354 and are exemplified in the pending application at page 115 for Exhibit 4, pages 126 and 134 for Exhibit 5, and page 135 for Exhibit 6. These photographs all demonstrate healthy surviving insecticidal tomato plants, capable of producing fruit (see Exhibit 7) and capable of being used to produce succeeding generations of plants by normal

methods of sexual reproduction. All plants are the result of stable transformation and incorporate a foreign insecticidal protein gene that is expressed.

18. Exhibit 8 is a report about a field trial of insect resistant tomato plants made according to the methods of the invention. Preliminary laboratory screening protocols are described on the first page. Forty plants out of 550 laboratory tested plants were chosen for field testing. The report describes the field bioassays on page 2, the monitoring and control procedures on page 3, and the summary of morphology and fruit yield of the plants in total numbers and pounds on pages 3-5.

19. Exhibit 9 provides a photograph and related notebook pages related to transgenic potato plants, produced according to the methods of the invention, that have been maintained in the greenhouse for a number of years and have been periodically cut back as is apparent in the exhibit. In the photograph, control nontransformed Russet Burbank potato plant is on the left with a tuber derived transgenic experimental plant 0209-2 on the right. Plant 0209-2 resulted after transformation with a vector having a CaMV 35S promoter/Alfalfa Mosaic Virus leader sequence/Bt insecticidal protein gene [HD-1 (Dipel) 5.3 kb class gene], which plant was produced according to the teachings of the first application at pages 20-81. The accompanying two notebook pages in this exhibit describe an ELISA analysis for insecticidal protein expression in the plants where the ELISA data for the 0209-2 potato was positive but the control plant was negative. The photographs demonstrate the health and vigor of the transgenic Russet Burbank potato expressing an insecticidal protein gene which can be compared to the non-transformed control plant.

20. Exhibit 10 provides a photograph with accompanying related note book pages of three transgenic coker 201 cotton seeds and fiber derived from a cross between a normal parental coker 201 cotton plant and a second parental coker 201 cotton plant transformed with pSU605 and produced according to the methods of the invention. The pSU605 vector was originally named pH 575-S-B. The first notebook page provides the bioassay data for the parent plant which indicates the toxicity to insects of the transgenic cotton leaves of parent plant #2 [pH 575-S-B-2 (El^+)]. The accompanying four additional notebook pages provide the ELISA data indicating that plant 575S-b-2 has a positive ELISA and was the 14th plant sampled in the test. The parent plant was produced by transformation with a vector containing a small subunit promoter with a full length HD-73 insecticidal protein gene according to the teachings of the first application at pages 20-81.

21. Exhibit 11 are notebook pages demonstrating the characterization of sunflower tumors having positive ELISA results in an experimental analysis of sunflower transformed with a Bt insecticidal protein gene vector for expression of the insecticidal protein. The sunflower tumors were produced after transformation of the vector according to the teachings of the first application at pages 20-81.

22. Exhibit 12 are notebook pages demonstrating the positive ELISA data in an analysis of Black Mexican Sweet Corn callus transformed with a coleopteran specific Bt insecticidal protein gene vector. The callus was produced using the methods of the invention according to the teachings of the first application at pages 20-81 to produce the vector for the transformation and the callus tissue resulting from the transformed plant cells.

23. Based on all the foregoing evidence related to the development of many species and varieties of healthy, stably transformed transgenic plants, tissues and cells expressing foreign insecticidal proteins according to the methods of the invention and my knowledge in this scientific area, it is my belief and opinion that Dr. Michael Adang and Dr. John Kemp invented insect resistant plants and that it is through their efforts that such novel and useful plants, tissue and cells were first developed.

24. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the captioned patent.

Dated: April 2, 1992

GUY A. CARDINEAU